

Mapping serotonergic dysfunction in MDMA (ecstasy) users using pharmacological MRI

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA or ecstasy) is a popular recreational drug that has been shown to induce loss of brain serotonin (5-HT) neurons. The purpose of this study was to determine the usefulness of pharmacological magnetic resonance imaging (phMRI) in assessing 5-HT dysfunction by examining the hemodynamic response evoked by infusion with the selective 5-HT reuptake inhibitor citalopram. We studied the effects of MDMA on brain hemodynamics using arterial spin labeling (ASL) based phMRI following a citalopram challenge (7.5 mg/kg, i.v.), combined with [¹²³I]β-CIT SPECT imaging in ten male MDMA users and seven healthy non-users. Single photon emission computed tomography (SPECT) imaging was used to assess the availability of 5-HT transporters (SERT). Imaging results were compared with the results of behavioral measures and mood changes following drug administration, in both groups (using the Beck Depression Inventory, Barratt Impulsiveness Scale and a visual analog scale). Reductions in SERT binding were observed in the occipital cortex of MDMA users. In line with this, citalopram induced decreases in cerebral blood flow (CBF) in the occipital cortex of MDMA users. ASL based phMRI also detected a CBF decrease in the thalamus of MDMA users. In concordance with imaging findings, behavioral measures differed significantly between MDMA users and controls. MDMA users had higher impulsivity scores and felt more uncomfortable after citalopram infusion, compared with control subjects. Our findings indicate that phMRI is very well suited for in-vivo assessment of 5-HT dysfunction. © 2011 Elsevier B.V. and ECNP. All rights reserved.

1. Introduction

Disturbances of the serotonergic (5-HT-ergic) system have been linked to several neuropsychiatric disorders. The serotonin transporter (SERT) is located on the presynaptic membrane of 5-HT neurons and plays a crucial role in the reuptake of 5-HT and in modulating 5-HT concentrations in the synaptic cleft.

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Typically, the central 5-HT system has been visualized by single photon emission computed tomography (SPECT) and positron emission tomography (PET), using ligands for SERT or 5-HT receptors. Several groups observed reduced SERT binding in recreational 3,4-methylenedioxymethamphetamine (MDMA) users, by using SPECT or PET imaging (de Win et al., 2008a; McCann et al., 2005; Reneman et al., 2001). SPECT and PET imaging however, depend on the use of radioactive tracers, rendering them less suitable for pediatric studies or for repeated measurements.

A promising technique to investigate the 5-HT system is pharmacological magnetic resonance imaging (phMRI). In phMRI, a neurotransmitter specific pharmacological challenge is given, causing changes in neurovascular coupling and subsequent region specific hemodynamic changes. The selective serotonin reuptake inhibitor (SSRI) citalopram increases 5-HT release by inhibiting the reuptake of 5-HT. It has been used previously in phMRI studies and has been proven an adequate probe of 5-HT function (Attenburrow et al., 2001; Seifritz et al., 1996; Anderson et al., 2007; McKie et al., 2005; Rose et al., 2006; Wingen et al., 2008). Whereas in previous studies, the blood oxygenation level dependent (BOLD) signal was used to visualize hemodynamic changes, nowadays perfusion MRI by arterial spin labeling (ASL) is also more frequently used (Chen et al., 2011; Hagino et al., 1998; Ogawa et al., 1990; Rao et al., 2000; Khalili-Mahani et al., 2011). ASL is a non-invasive perfusion imaging modality that uses magnetically labeled blood water protons as an endogenous tracer of cerebral blood flow (CBF). By subtracting labeled and unlabeled images, a CBF map is generated, which can be used for visualization and quantification of CBF (Golay et al., 2004) with sufficient precision (Gevers et al., 2011). It has been shown that ASL based phMRI gives a more precise measurement of pharmacologically induced hemodynamic changes (Tjandra et al., 2005).

MDMA itself has already been used in phMRI to assess its effects on the 5-HT system (Brevard et al., 2006). Brevard et al. (2006) suggested in their discussion that MDMA-induced 5-HT neurotoxicity would probably be an adequate model to test the applicability of phMRI in assessing 5-HT dysfunction. McKie et al. (2005) already investigated 5-HT function in healthy humans using BOLD based phMRI. The usefulness of phMRI in assessing 5-HT dysfunction in humans, however, has not yet been studied.

The purpose of this study was to determine whether ASL based phMRI is a useful method in assessing 5-HT dysfunction by examining hemodynamic responses evoked by citalopram infusion in MDMA users and healthy controls. The results of ASL based phMRI with an intravenous citalopram challenge were combined with SPECT imaging and behavioral measures. Thus, comparisons could be made between imaging findings, measures of SERT binding and the behavioral consequences of MDMA-induced changes to the serotonergic system. We hypothesized that in MDMA users we would observe a reduction in hemodynamic response evoked by citalopram, in brain regions with reduced SERT densities, as citalopram binds highly selective to the SERT.

2. Experimental procedures

2.1. Subject recruitment and study design

Subjects were recruited by advertisements and word of mouth. After written informed consent, 10 male MDMA users and 7 healthy male

controls were included in the study. The eligibility criterion for the MDMA group was previous use of more than 50 tablets of MDMA. The cutoff point of 50 tablets was based on previous studies (Reneman et al., 2001). The 7 control subjects were healthy subjects with no self-reported prior use of MDMA. Participants agreed to abstain from use of MDMA and other psychoactive drugs for at least 2 weeks before the studies and were asked to undergo urine drug screening on the days of assessment (with an enzyme-multiplied immunoassay for amphetamines, including MDMA). Exclusion criteria were: a positive drug screen, any neuropsychiatric diagnosis or history of brain disease or injury, use of medication with affinity for SERT (e.g., SSRIs), or any contra-indication to MRI such as metallic implants or claustrophobia. The local institutional Medical Ethics Committee approved the study.

2.2. Behavioral measures

We selected two widely used tests for assessment of mood and impulsivity, as 5-HT plays a central role in mood regulation and impulsivity: the Beck Depression Inventory (BDI, Beck, 1961) was administered as a self-report estimate of mood and the Barratt Impulsiveness Scale (BIS version 11, Patton et al., 1995) as a self-report estimate of impulsivity.

In addition, drug effects and mood changes following citalopram infusion were assessed using simulated visual analog rating scales (VAS) 5 min before and 5 min after scanning, rating on a scale of 1 to 10 for anxious feelings, tension, feeling comfortable or uncomfortable, light-headedness, wakefulness, drowsiness and nausea.

2.3. Procedures

Subjects underwent phMRI followed by SERT SPECT after a two to four week interval (range 17–31 days, mean 21.7 ± 5.2 days). For the phMRI scan, 7.5 mg citalopramhydrochloride obtained from Lundbeck GmbH (Hamburg, Germany), was diluted in 45 mL of saline solution. The drug was infused after 7.5 min of baseline scanning over a period of 7.5 min in order to examine the effects of citalopram on the hemodynamic response. This timeframe was based upon previous studies investigating the hemodynamic effects of citalopram infusion (McKie et al., 2005). Post-infusion imaging was performed during 22.5 min, resulting in a total scanning duration of 37.5 min.

2.4. MR imaging

All MR imaging was performed using a 3.0 T Philips MR scanner equipped with a SENSE 8-channel head coil and body coil transmission (Philips Medical Systems, Best, The Netherlands). Each session protocol consisted of a high resolution 3DT1-weighted anatomical scan for registration and segmentation purposes and a phMRI sequence. For the phMRI sequence, a pulsed ASL sequence was used, that was based on the PULSAR sequence developed by Golay (Golay et al., 2005). phMRI imaging parameters were: TR/TE 3000/14 ms; FOV 240×240 mm²; matrix size 80×79 ; 17 slices; thickness 7 mm; no gap; gradient echo single shot EPI; SENSE 2.0; post-labeling delay 1.2 to 2 s; number of dynamics 375. One dynamic consisted of a control and a labeled image. The labeling plane was positioned parallel to the imaging volume with a labeling gap between the center of the imaging volume and the labeling volume of 90 mm. We acquired 75 dynamics at baseline, 75 dynamics during infusion and 225 dynamics post-infusion.

2.5. MR processing

FSL 4.1 (FMRIB-Software-Library, Functional Magnetic Resonance Imaging of the Brain Center, Dept. of Clinical Neurology, University of Oxford, Oxford, UK, <http://www.fmriv.ox.ac.uk>), the SPM toolbox, Freesurfer image analysis suite, (<http://surfer.nmr.mgh.harvard>).

edu/) and Matlab (The MathWorks Inc., Natick, USA) were used for off-line data processing.

Subtraction of labeled and control ASL images yielded whole brain perfusion weighted images. The mean equilibrium magnetization (M_0) of arterial blood per subject was calculated, from which the absolute CBF was computed following the methods outlined by Chalela et al. (2000). The resulting ASL images were averaged over 25 volumes (2.5 min) to increase the signal-to-noise ratio (SNR). This resulted in 15 separate time bins.

3DT1 anatomical scans were segmented into gray and white matter. Perfusion weighted images were transformed into anatomical space by an affine registration of the mean volumes per time bin to the gray matter masks of corresponding anatomical scans. An isotropic resampling of 3.0 mm was chosen.

The structural images were non-rigidly normalized to a population-based average using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) (Ashburner, 2007), to allow for a voxel-based analysis. The non-rigid transformations were applied to the ASL volumes that were previously registered to gray matter masks, such that all dynamics of all subjects resided in one common frame of reference. Finally, a Gaussian smoothing with FWHM = 6 mm was applied to all volumes (Fig. 1).

2.6. SPECT imaging

The subjects were examined using SPECT with the SERT ligand 123I-labeled 2 β -carbomethoxy-3 β -(4-iodophenyl)tropane ([123I] β -CIT). SPECT images were obtained in 10 MDMA users and 7 healthy controls, however one SPECT scan had to be excluded due to poor image quality. 123I-labeled β -CIT was prepared as previously described (Reneman et al., 2001). Potassium iodide was used to block thyroid uptake of free radioactive iodide. SPECT images were acquired with a brain dedicated SPECT system (Neurofocus, an update of the Strichman Medical Equipment 810X, Strichman Medical Equipment Inc., Medfield, Mass., USA). This is a 12-detector single-slice scanner with a full width at half maximum resolution of 6–7 mm. The acquisition of SPECT images was the same as previously described (de Win et al., 2005) and was commenced 4 h after i.v. injection of approximately 112 MBq (3.02 mCi) [123I] β -CIT since at this time peak specific binding to SERTs has been reached (Pirker et al., 2000).

2.7. SPECT processing

Reconstruction and attenuation correction of all images were performed as previously described (Booij et al., 1997). To be able to correct the image for background radioactivity (non-specific binding) a predefined ROI was placed over the cerebellum. The ROI was placed on the three consecutive SPECT slices with the highest counts. For analysis, the Statistical Parametric Mapping software (SPM 8 Wellcome

Department of Imaging Neuroscience, Functional Imaging Laboratory, London, UK; <http://www.fil.ion.ucl.ac.uk/spm>) was used. All scans were realigned to an arbitrarily chosen subject, after which an average template was calculated. Subsequently, the scans were co-registered and normalized to this template. The registered scans were intensity-scaled to the corresponding mean cerebellar non-specific counts per voxel, obtained from the ROI analysis (de Win et al., 2008b).

3. Statistical analysis

All data were analyzed in SPSS version 17.0 (SPSS Inc, Chicago, IL) and are presented as mean \pm SD unless otherwise indicated.

3.1. Characteristics of the sample and behavioral measures

Differences in continuous variables were analyzed using unpaired 2-tailed student's t-tests unless stated otherwise and ordinal data were compared using chi-square tests.

3.2. SPECT and pHMRI studies

Analysis of SPECT and pHMRI datasets was carried out using a voxel-based analysis.

Unpaired t-testing was used to compare differences between groups to evaluate the overall group effects ASL and SPECT datasets. The chance of a type I error was set at 0.01 to correct for multiple comparisons.

Paired t-testing was used to compare differences within groups, to analyze the effects of the citalopram challenge in the ASL datasets. Weights were chosen to represent a post-infusion increase [–1, 0, 1] or decrease [1, 0, –1]. By applying this statistical model to ASL pHMRI data, the response to the challenge was determined for both healthy controls and MDMA users. In addition, the interaction between group and challenge effects was also calculated, to determine to what extent groups reacted differently to the challenge.

The SPECT datasets were analyzed as previously described (de Win et al., 2008a). A minimal cluster size of ten voxels was maintained, besides the significance threshold of 0.01, to correct for multiple comparisons.

4. Results

Group characteristics are presented in Table 1. Controls and users of MDMA differed slightly but statistically significantly in age ($p=0.02$). Mean cumulative dose of MDMA was 281 tablets and time since last dose 2.1 months. Although the MDMA users had used on average more recreational drugs such as alcohol, amphetamine and cocaine, this difference was not statistically significant.

4.1. Behavioral measures

BIS scores were significantly higher for users of MDMA than control subjects (74.0 ± 8.7 vs. 54.3 ± 9.1 , $p=0.02$), but we did not observe a group difference in BDI scores (5.1 ± 5.6 vs. 2.7 ± 2.5 ; n.s.).

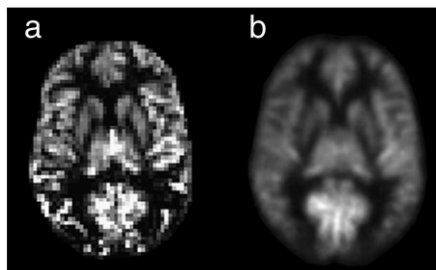


Figure 1 (a) An axial slice of the perfusion measured with ASL, averaged over time in 375 dynamics. (b) Averaged ASL-image over subjects after non-rigid normalization (see text). Notice the preservation of anatomical detail, suggesting good alignment between subjects.

Table 1 Characteristics of drug abuse and alcohol exposure.

	MDMA n = 10	Controls n = 7 ^a	p-value
Age	25.4 (±2.1)	21.3 (±3.9)	0.02
Last 3 months use of Alcohol, no. times used			NS ^b
None	1	0	
10–19	0	2	
20–39	4	3	
> 40	5	1	
Tobacco, no. cigarettes			NS ^b
None	5	6	
1–5 cig./day	2	0	
6–20 cig./day	2	0	
> 20 cig./day	1	0	
Cannabis, no. times used			NS ^b
None	4	5	
1–5	3	0	
10–39	2	1	
> 40	1	0	
Amphetamine, gr	0.3±0.7	0±0	0.16 ^c
Cocaine, no. lines	0.8±1.6	0±0	0.21 ^c
<i>Characteristics of ecstasy use</i>			
Duration of use (years)	6.5±3.1		
Lifetime exposure (tot. no. tablets)	281±250.97 [range 50–900]		
Usual dose (no. tablets/occasion)	3.65±3.08		
Time since last tablet (months)	2.14±2.34		

^a Drug history questionnaire missing for 1 control subject.^b Chi-square test.^c Log transformed.

Overall, citalopram infusion was well tolerated, apart from one of the MDMA users experiencing an acute increase in lightheadedness and nausea short after infusion.

VAS scores showed statistically significant decreases in tension in the control subjects ($p < 0.01$) as well as feeling less sweaty and

clammy ($p = 0.02$ and $p = 0.05$) when comparing pre- to post-infusion (Table 2). MDMA users were more lightheaded before infusion ($p = 0.03$) when compared to control subjects, but other parameters did not statistically differ from controls before citalopram infusion. After infusion, MDMA users felt significantly more

Table 2 Behavioral measures.

	Controls		MDMA users		p-value	
	Mean (SD)		Mean (SD)			
BDI	2.7 (±2.5)		5.1 (±5.6)		n.s.	
BIS	54.3 (±9.1)		74.0 (±8.7)		<0.01	
VAS	Controls		MDMA users		Controls vs. MDMA users	
	Δpost–pre (SD)	p-value	Δpost–pre (SD)	p-value	p-value pre	p-value post
Alert	–1.1 (1.9)	n.s.	–0.8 (2.3)	n.s.	n.s.	n.s.
Tense	–2.3 (1.4)	0.005	–0.5 (1.9)	n.s.	n.s.	n.s.
Calm	0.3 (1.4)	n.s.	–0.9 (1.6)	n.s.	n.s.	n.s.
Clammy	–0.7 (0.8)	0.05	–0.5 (2.0)	n.s.	n.s.	n.s.
Comfortable	0.4 (1.0)	n.s.	–1.4 (2.9)	n.s.	n.s.	0.02
Nausea	0 (0)	n.a.	0 (0.9)	n.s.	n.s.	n.s.
Sleepiness	1.1 (1.6)	n.s.	1.0 (1.8)	n.s.	n.s.	n.s.
Light-headedness	0.7 (1.6)	n.s.	1.6 (2.8)	n.s.	0.03	0.02
Sweaty	–2.1 (1.6)	0.02	–1.2 (2.6)	n.s.	n.s.	n.s.
Uncomfortable	–0.3 (0.8)	n.s.	0.5 (2.7)	n.s.	n.s.	0.05

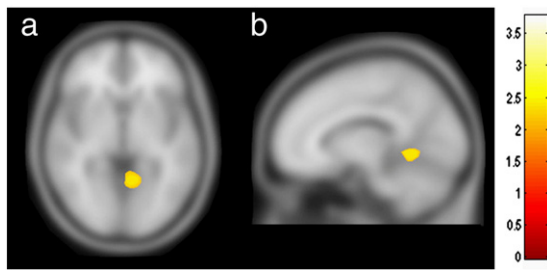


Figure 2 Statistical parametric map of $[^{123}\text{I}]\beta\text{-CIT}$ SPECT binding ratios. The yellow cluster represents statistically significant lower binding SPECT ratios in the left anterior occipital lobe of MDMA users when compared to controls in the axial (a) and sagittal (b) plane. The image is superimposed on a T1 anatomical atlas from SPM. Scale bar indicates z-scores. Statistic images were thresholded with a cluster significance threshold of $p=0.01$.

lightheaded ($p=0.02$), uncomfortable ($p=0.02$) and tense ($p=0.04$) than controls.

4.2. SPECT imaging

SERT binding ratios were significantly reduced in the left anterior part of the occipital lobe of MDMA users when compared to controls (Fig. 2).

4.3. ASL based pHMRI

4.3.1. Challenge effect

Administration of citalopram to control subjects did not result in marked CBF changes compared to baseline (with a $p=0.01$

threshold). MDMA users showed a statistically significant CBF decrease in response to the 5-HT challenge that was most prominent in the left thalamus (Fig. 3 panel A). In addition, a CBF decrease was observed in the right occipital cortex and in the right frontal cortex. Also, a significant CBF increase was noted in the left globus pallidus and left frontal cortex (Fig. 3 panel B).

4.3.2. Group effect

Baseline mean whole brain CBF in MDMA users was significantly higher in MDMA users than in controls (mean users 49.4 ± 9.1 mL/100 g/min vs. mean controls 44.1 ± 9.4 mL/100 g/min, $p<0.01$, see Fig. 4). However, there was no whole brain response to the citalopram challenge on the CBF measurements.

When comparing the changes in CBF before and after the challenge (ΔCBF) between groups, the ΔCBF in MDMA users in response to the 5-HT challenge differed statistically significantly from the ΔCBF in controls. This ΔCBF (a decrease) was present particularly in the left thalamus and several regions in the bilateral anterior occipital lobe of MDMA users (Fig. 5). No CBF increases in response to the challenge were observed when comparing the two groups.

5. Discussion

The most important findings of our study are threefold. Firstly, we observed a statistically significant CBF decrease in the occipital cortex of male MDMA users in response to a 5-HT challenge with the SSRI citalopram. SPECT imaging revealed statistically significant lower SERT binding ratios in the occipital cortex of male MDMA users when compared to control subjects. Secondly, ASL based pHMRI showed

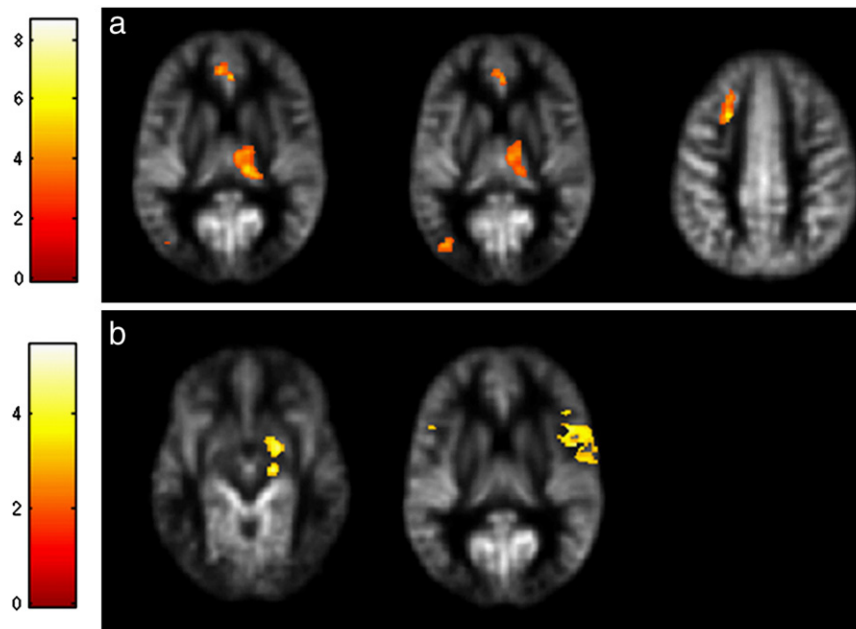


Figure 3 Statistical parametric map of CBF change induced by 7.5 mg citalopram in MDMA users. Effect of the challenge: MDMA users show both a decrease (a) and increase (b) in CBF in response to the challenge. A decrease in CBF was noted in the thalamus, right frontal and right occipital cortex (panel A). The significant cluster of decreased CBF in the prefrontal cortex/cingulate gyrus is located close to the central sulcus and probably reflects remaining intravascular label and is not to be considered a true challenge effect. An increase in CBF was also observed in the thalamus, in addition to the left globus pallidus and frontal cortex (Panel B). Scale bar indicates z-scores. Statistic images were thresholded a cluster significance threshold of $p=0.01$.

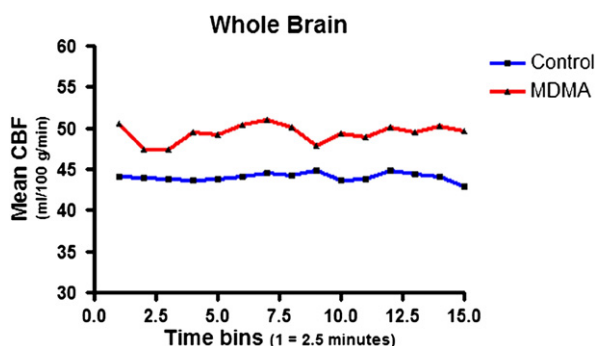


Figure 4 Whole brain CBF data in MDMA users and healthy controls indicating statistically significant higher mean CBF values in users vs. controls during the entire scanning period.

significantly higher mean whole brain CBF in MDMA users than in controls. Also, ASL based phMRI showed a significant group effect in the left thalamus that was not observed in the SPECT study. Thirdly, MDMA users had higher impulsivity scores on a standardized impulsivity scale which correlated with SERT densities and hemodynamic changes in the occipital cortex. Furthermore, they felt significantly more uncomfortable after the citalopram infusion compared to the control subjects. The combination of imaging findings and behavioral measures suggests that MDMA-induced changes to the serotonergic system can be visualized using phMRI.

Only one other study has exclusively studied *male* MDMA users with $[123\text{I}]\beta\text{-CIT}$ SPECT (Semple et al., 1999). Similar to that study, we observed lower SERT binding in the occipital lobe of male MDMA users when compared to controls (Semple et al., 1999). The occipital cortex seems particularly sensitive to effects of MDMA because it shows the largest reductions in SERT densities in human and experimental studies (Kish et al., 2010; McCann et al., 2008; Szabo et al., 2002). In moderate users, reductions have only been observed in parieto-occipital and occipital cortex (Reneman et al., 2001).

To support the SPECT findings of this study performed in a relatively small population, we reanalyzed $[123\text{I}]\beta\text{-CIT}$ SPECT of 21 male MDMA users (mean age 26.6 years) and 6 male controls (29.2 years), who participated in a previous study of

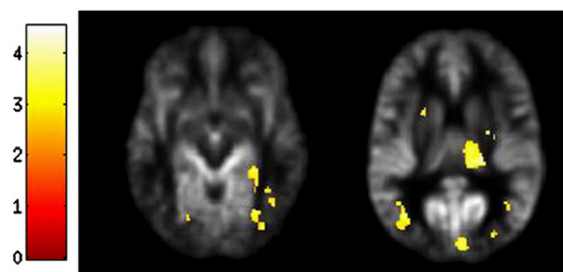


Figure 5 Statistical parametric map of CBF changes induced by 7.5 mg citalopram in MDMA users compared to control subjects. Group effect: Statistically significant lower CBF in the left thalamus, and bilateral occipital lobe in response to a citalopram injection in MDMA users when compared to healthy controls superimposed on the time- and subject-averaged CBF. Scale bar indicates z-scores. Statistic images were thresholded a cluster significance threshold of $p=0.01$.

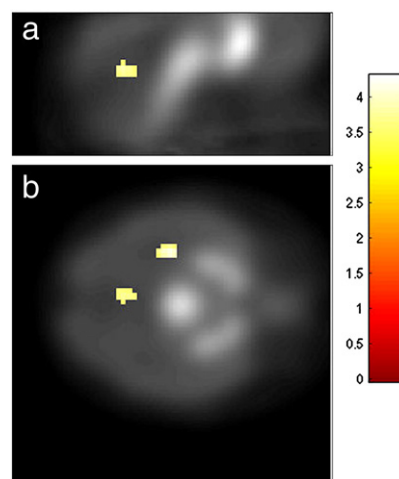


Figure 6 Statistical parametric map of $[123\text{I}]\beta\text{-CIT}$ SPECT binding ratios. The yellow cluster represents statistically significant lower binding SPECT ratios in the right parieto-occipital and anterior-occipital lobes of MDMA users when compared to controls in the sagittal (a) and axial (b) plane. The image is superimposed on the mean SPECT scan from all subjects. Scale bar indicates z-scores. Statistic images were thresholded with a cluster significance threshold of $p=0.001$.

our group (Reneman et al., 2001) using the same methods as described in the current study. MDMA users had used on average 487 tablets (± 679), whereas controls had never been exposed to MDMA. In this larger sample of male MDMA users with a (much) higher MDMA exposure, voxel-based analysis confirmed our present findings of reduced SERT binding ratios in the anterior occipital cortex (see Fig. 6), in addition to the parieto-occipital cortex. Our current SPECT findings involving 'only' a specific brain region of MDMA users are thus in concordance with literature and previous data of male MDMA users. Other studies involving both male and female MDMA users observed reductions in SERT also in other brain regions (e.g., Reneman, 2001; McCann et al., 1998; de Win et al., 2008a). Therefore, it has been suggested that women are more sensitive than men to MDMA-induced reductions in SERT binding (Buchert et al., 2004; Reneman et al., 2001).

We used the SSRI citalopram as a pharmacological challenge. One could argue on the choice for this SSRI, since it might also induce a general vascular response. However, McBean et al. (1999) demonstrated in rats that local CBF changes in response to a citalopram challenge are correlated to changes in glucose metabolism rather than reflecting a general vascular response. Furthermore, in higher doses citalopram does not alter cardiovascular parameters (Penttilä et al., 2001; Seifritz et al., 1996). In the current study, citalopram evoked hemodynamic changes in a number of specific brain regions in MDMA users, but not in control subjects. The pattern of hemodynamic changes appeared to be related to the release of 5-HT, as the strongest hemodynamic changes were observed in cortical regions and subcortical gray matter (areas such as putamen and thalamus, with a high SERT density in healthy subjects), and not as much a general vascular response.

As hypothesized, we observed a reduced hemodynamic response in the occipital cortex of MDMA users with SERT

reductions. We hypothesized that a reduction in hemodynamic response in brain regions with reduced SERT densities would reflect reduced 5-HT transmission and thus reduced 5-HT mediated changes in neuronal activity. There is indeed accumulating evidence that reductions in hemodynamic responses reflect decreases in neuronal activity. Preece et al. (2009) have shown region specific increases in BOLD signal evoked by the 5-HT releaser fenfluramine. Interestingly, pretreatment with the inhibitor of 5-HT synthesis p-chlorophenylalanine attenuated this BOLD response, suggesting that the BOLD signal is sensitive to increased availability of 5-HT. Jenkins et al. (2004) performed an experiment similar to ours, investigating the dopaminergic system in 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys using pHMRI. They observed a reduction in regional cerebral blood volume evoked by the dopamine releaser amphetamine.

This response correlated well with decreases in dopamine transporter availability. The fact that ASL based pHMRI detected CBF changes in a brain region that was also identified by significant differences in SPECT binding and previously mentioned in literature, suggests that pHMRI is a powerful tool for assessment of 5-HT dysfunction (Kish et al., 2010; McCann et al., 2008; Reneman, 2001; Semple et al., 1999; Szabo et al., 2002).

Interestingly, we observed a 12% higher overall CBF in MDMA users. This observation is in line with previous studies, in which 5-HT has been described to modulate cerebral perfusion. It has been suggested that after the initial excessive release of 5-HT induced by MDMA, normalization or even depletion of 5-HT at a later time point results in an increase in CBF, due to MDMA-induced loss of 5-HT neurons (Chang et al., 2000; Reneman et al., 2000).

ASL based pHMRI showed an additional brain region (the thalamus) in which a group difference was detected, unlike SPECT. Besides the occipital cortex, the thalamus is a brain region that is particularly sensitive to MDMA's effects (de Win et al., 2008b; McCann et al., 2005, 2008). Therefore, ASL based pHMRI seems very well suited for assessing 5-HT dysfunction. It has been reported that ASL is not hampered by signal drifts, as it measures one single physiological parameter (CBF) and has a greater inter-subject reliability compared to BOLD (Tjandra et al., 2005). If ASL pHMRI is a better technique than BOLD pHMRI in evaluating 5-HT dysfunction, remains to be assessed.

BIS impulsivity scores were higher in users of MDMA than in control subjects. This observation was described previously by our study group in a prospective study cohort (de Win et al., 2006). This indicates that the increase of impulsivity scores is at least partly due to MDMA use rather than a pre-existent personality trait in users, even after exposure to only a few tablets of MDMA. Interestingly, MDMA users also felt more uncomfortable after citalopram infusion when comparing to control subjects who felt more comfortable, providing further evidence that MDMA users and control subjects in this study not only differ in 5-HT mediated changes in neuronal activity, but also in behavior. The significant difference in impulsivity scores and the perception of drug effects and mood changes following citalopram infusion are in line with the challenge effects that were observed pHMRI and reduced SERT density in users observed by SPECT.

Several limitations of this study should be recognized. First of all, the sample size of the control group is relatively small. The results from the SPECT data however, are in accordance

with previous findings. For this reason, the pHMRI results should be replicated in larger groups to confirm our findings. Secondly, the groups we studied differed slightly but significantly in age. In literature, different values for the variability of 5-HT receptor and transporter availability with age have been reported. Generally, it seems that the availability of 5-HT transporters differs greatly between subjects. This difference exceeds the difference that would be introduced by the mean age difference present in our study population (Buchert et al., 2006; Parsey et al., 2002; Ryding et al., 2004). In line with this, we did not observe a significant effect of age on SERT binding ($p=0.87$), and the results of our study were not affected by introducing age as a covariate: the effect of group remained significant ($p=0.03$). Thirdly, in this cross sectional study design, we had to rely upon the self-reported use of drugs of abuse and alcohol. Theoretically, the present findings could be due to other drugs than MDMA, since MDMA users had more experience with other recreational drugs. However, there was no significant difference in the use of other recreational drugs between groups. Fourthly, one might ascribe reductions in occipital SERT to direct pharmacological effects. This is unlikely however, since participants had to abstain from psychoactive drugs for at least two weeks before data acquisition. The latter was screened for by urine drug screening at the day of examination. Thirdly, it is possible, that pre-existing differences between MDMA users and controls underlie differences in SERT densities and brain activity evoked by citalopram. However, previous longitudinal neuroimaging studies only observed changes in the thalamus of incidental MDMA users (de Win et al., 2008b). Finally, the fact that ASL pHMRI did not reveal any citalopram response in our controls could be attributable to the spatial resolution with which ASL data were acquired. We did however observe clear responses to citalopram in MDMA users, indicating that responses in this group were large enough to overcome possible resolution issues. Moreover, imaging alterations in users were in concordance with behavioral measures in this group. Therefore, we believe that the results of the current study clearly show the effects of MDMA on the brain and that these effects can be imaged by ASL based pHMRI.

In conclusion, this study provides evidence that citalopram pHMRI is a powerful new and non-invasive tool for assessment of 5-HT dysfunction. We observed a reduction in hemodynamic response in a brain region with reduced SERT densities, presumably reflecting 5-HT mediated changes in neuronal activity. MDMA users and control subjects differed in 5-HT mediated changes in neuronal activity, a finding that was confirmed by the difference in behavioral measures between both groups.

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Contributors

Liesbeth Reneman designed the study and wrote the protocol. Sanna Gevers and Marieke Schouw collected the data, performed part of the analysis and wrote the article. Aart Nederveen designed the

MRI scanning protocols and supervised part of the MRI analysis. Matthan Caan performed and supervised part of the MRI and SPECT analysis. Jan Booij supervised and coordinated the SPECT imaging procedures. Charles Majoie supervised and advised on MRI imaging procedures. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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